Reference: *Biol. Bull.* **206:** 13–24. (February 2004)

Identification of Juvenile Hormone-Active Alkylphenols in the Lobster *Homarus americanus* and in Marine Sediments

WILLIAM J. BIGGERS[1] AND HANS LAUFER[2,*]

[1] *Department of Biology, Wilkes University, Wilkes-Barre, Pennsylvania 18766; and* [2] *Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543*

Abstract. We have identified, by gas chromatography/mass spectrometry, four alkylphenols that are present in the hemolymph and tissues of the American lobster *Homarus americanus* and in marine sediments. These alkylphenols are used industrially in antioxidant formulations for plastic and rubber polymer manufacturing, and are similar in structure to a known endocrine disruptor, bisphenol A. The compound 2-*t*-butyl-4-(dimethylbenzyl)phenol was present at concentrations of 0.02 to 1.15 μg/ml in hemolymph and 8.95 to 21.58 μg/g in sediments. A second compound, 2,4-bis-(dimethylbenzyl)phenol, was present at concentrations between 0.07 and 19.78 μg/ml in hemolymph and 138.94 to 224.89 μg/g in sediment, while a third compound, 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, was found at concentrations between 0.01 and 13.00 μg/ml in hemolymph, 2.55 and 6.11 μg/g in hepatopancreas, and 47.85 and 74.66 μg/g in sediment. A fourth compound, 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol, was found at concentrations of 0.20 to 70.71 μg/ml in hemolymph, 23.56 to 26.89 μg/g in hepatopancreas, and 90.68 to 125.58 μg/g in sediment. These compounds, along with bisphenol A, 4-dimethylbenzylphenol, and nonylphenol, display high juvenile hormone activity in bioassays. Alkylphenols at high concentrations are toxic to crustaceans and may contribute significantly to lobster mortality; at lower concentrations, they are likely to have endocrine-disrupting effects.

Received 2 July 2003; accepted 17 November 2003.

* To whom correspondence should be addressed. E-mail: laufer@uconn.edu

Introduction

The lobster population in western Long Island Sound has been decimated in recent years, and a variety of factors have been implicated, including elevated temperatures, anoxia, paramoeba infestation, and exposure to pesticides and other chemicals entering the marine environment (Long Island Sound Lobster Health Symposium, 2003). Since crustacean reproduction, development, and metamorphosis are known to be partly regulated by a juvenile hormone, methyl farnesoate (MF) (reviewed by Laufer and Biggers, 2001), we have joined these investigations, asking whether lobsters and marine sediments contain exogenous chemicals with juvenile-hormone activity (JH activity) that may affect the health of these crustaceans and act as endocrine disruptors.

MF is a sesquiterpenoid hormone that is similar in structure to insect juvenile hormones and is secreted by the mandibular organ. Previous investigations in our laboratory indicated that extracts of marine organisms and sediments display JH activity in insect cuticle bioassays (Biggers and Laufer, 1992; Biggers, 1994). Here we report the identification of four alkylphenols that are similar in structure to the endocrine disruptor bisphenol A and are present in sediment samples. We found these alkylphenols to possess high JH activity, and one of them was initially developed as a mosquito insecticide. We also report finding these four alkylphenols in lobster hemolymph and two alkylphenols in hepatopancreas tissue. These results suggest that these chemicals, like bisphenol A and other alkylphenols, may have widespread distribution in the marine environment where, at low concentrations, they may be acting as endocrine disruptors in the lobster, and at higher concentrations,

they may contribute to the high mortalities of lobsters in Long Island Sound and elsewhere.

Materials and Methods

Animals

Mature male and female lobsters, ranging in weight between 300 and 450 g (shorts) were collected from Long Island Sound and from Vineyard Sound, Massachusetts. Long Island Sound animals were kept in recirculating tanks at Storrs, Connecticut, and animals from Vineyard Sound were maintained in running seawater at the Marine Biological Laboratory, Woods Hole, Massachusetts. The animals were fed squid *ad libitum*. Immediately upon arrival, the animals were bled with 5-cc plastic syringes and 23-gauge needles.

Chemicals

The following chemicals were purchased from Sigma-Aldrich: 4-cumylphenol [also known as 4-dimethylbenzylphenol]; 2,4-bis-(dimethylbenzyl)phenol; bisphenol A [also known as 2,2-bis(4-hydroxyphenyl)propane]; nonylphenol-(mixed isomers); the juvenile hormones JH III and JH I; lutein; cholesterol; arachidonic acid; stearic acid; and farnesol. Pyriproxyfen was kindly provided by Dr. William Bowers (University of Arizona), and 2-*t*-butyl-4-(dimethylbenzyl)phenol, 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol, and *trans-trans* methyl farnesoate were synthesized in the laboratory. Acetonitrile, acetone, and hexane used for extractions and in HPLC analysis were purchased from Fisher Chemical and were of HPLC grade.

Extraction of marine sediments

Samples were taken from the top 6–12 in. of marine sediment in the intertidal zones of Vineyard Sound, Massachusetts, and Great Bay, New Jersey. The sediments were filtered through a 1-mm wire sieve to remove large debris, then kept frozen at −20 °C. A 25-g portion (wet weight) of each sediment sample was extracted with 50 ml of acetone, and the yellowish acetone extract was filtered through Whatman #1 filter paper to remove particulates. Five milliliters of the acetone filtrate was evaporated to dryness under nitrogen in glass vials and resuspended in 200 μl of hexane. The resuspended extract (200 μl) was applied to the top of a PrepSep silica solid-phase extraction column (Fisher Scientific) that had been pre-equilibrated with 10 ml of hexane. The columns were eluted sequentially with solvents of increasing polarity: 3 ml hexane; 3 ml hexane/20% ethyl ether; and finally, 3 ml 100% ethyl ether. The 100% ethyl ether eluate, which was yellow and showed high JH activity, was stored at −20 °C. For GC/MS analysis, 1 ml of the ethyl ether eluate was evaporated to dryness under nitrogen, then resuspended in 200 μl of hexane in small amber vials that were stored at −20 °C before analysis. By this method, using chemical standards, extraction and recovery efficiencies for the four phenols were 95% for 2-*t*-butyl-4-(dimethylbenzyl)phenol and 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, 47% for 2,4-bis-(dimethylbenzyl)-phenol, and 50% for 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol.

Extraction of hemolymph and hepatopancreas

For each hemolymph sample, 2 ml was added into Pyrex culture tubes (prebaked at 250 °C before use) containing 2 ml of cold 4% NaCl and 2 ml of acetonitrile, and then kept frozen at −20 °C. Samples were extracted with 2 ml of hexane by vortexing for 5 min. The tubes were centrifuged at 600 × *g* for 30 min, and 1.5 ml of the hexane phase was pipetted off and evaporated with a stream of nitrogen to 150 μl in amber glass vials. Before analysis by GC/MS, the hexane extracts were stored at −20 °C.

Hepatopancreas from two lobsters was rinsed in 4% NaCl and weighed; 2 g of the tissue was homogenized in 4 ml of cold acetonitrile and extracted with 4 ml of 4% NaCl and 2 ml of hexane. The hexane phases were then processed as described for the hemolymph samples before analysis by GC/MS. By this method, using standards, extraction and recovery efficiencies for the four phenols were 90% for 2-*t*-butyl-4-(dimethylbenzyl)phenol and 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, and 45% for 2,4-bis-(dimethylbenzyl)phenol and 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol.

Gas chromatography/mass spectrometry analysis

The lobster extracts and marine sediments were analyzed by gas chromatography/mass spectrometry using a Hewlett-Packard HP 5890 GC/5970 MSD GC/MS equipped with a 12.5-m, 0.20-mm-diameter column of cross-linked dimethylsilicone with film thickness of 0.33 μm. The operating conditions for GC were an initial temperature of 35 °C for 2 min, followed by a 15 °C/min ramp to 270 °C, then a 10-min hold at 270 °C, for a total run time of 27.67 min. Operating conditions for MS analysis were set to detect ion masses of 50 to 500 MW, by electron impact ionization using the scan mode. Individual compounds were identified by comparing their mass spectra with published library spectra. Identification of the four phenolic compounds and diisooctylphthalate was confirmed by comparison of mass spectra and retention times with those of chemical standards that were either purchased, as were 2,4-bis-(dimethylbenzyl)phenol and diisooctylphthalate, or synthesized in the laboratory, as was 2-*t*-butyl-4-(dimethylbenzyl)phenol, 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, and 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol described below. The phenols were quantified by integration of peaks and comparison of peak areas with known amounts of authentic standards.

Chemical synthesis

The compounds 2-*t*-butyl-4-(dimethylbenzyl)phenol and 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol were prepared by a Friedel-Crafts alkylation reaction. For synthesis, 15 g of 4-cumylphenol was dissolved in 75 ml of *t*-butylchloride in a 125-ml Ehrlenmeyer flask containing a stir bar. The reaction was started by addition of 100 mg of $FeCl_3$-$5H_20$ as catalyst with constant stirring at room temperature. The reaction was carried out in a fume hood to vent the evolved HCl gas. After 24 h, the reaction was stopped by transferring the reaction mixture to a 500-ml Ehrlenmeyer flask and adding 200 ml of distilled water. The phenols were extracted with 100 ml of hexane, and isolated and purified to 99% purity (determined by GC/MS) from the reaction mixture by normal-phase HPLC using a silica column (Varian microsorb, 250 mm, 100 A°) and hexane/6% acetone as running solvent.

The monophenol 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol was prepared using a Friedel-Crafts alkylation reaction, in the same way as were the other phenols, except that 15 g of 2,4-bis-(dimethylbenzyl)phenol was used as the starting material for synthesis, and the phenol product was purified by normal-phase HPLC using a running solvent of hexane/1% ethyl ether.

Bioassay for juvenile hormone activity

The phenolic compounds were assessed for JH activity using a rapid and sensitive assay based on their effects on the settlement and metamorphosis of larvae of the polychaete *Capitella* (Biggers and Laufer, 1996). The results of this bioassay are comparable to those of the *Galleria* JH bioassay: in both bioassays the test chemicals showed similar patterns of variation in JH activity. In this bioassay, no false positives or false negatives have been found for the compounds tested. Test chemicals were dissolved in 100% ethanol to give stock solutions of 0.1, 1, 10, and 100 μM, and aliquots of up to 100 μl of the stock solutions were added to 60-mm glass petri dishes each containing 10 ml of artificial seawater (Utikem, Co.), salinity 30 ppt, and 10 swimming 2-day-old metatrochophore larvae. Controls received up to 100 μl of ethanol. Dishes were swirled to disperse the test chemicals. Each concentration was tested in triplicate for every bioassay, and two bioassays were run (total dishes = 6, total number of larvae = 60) for each concentration tested. Settlement and metamorphosis of larvae was monitored using a stereobinocular microscope. After 1 h, the number of larvae that had settled and metamorphosed was recorded. Data are reported as EC_{50} values for each chemical, at the final concentration that induces 50% of the larvae to settle and metamorphose in 1 h.

Results

Alkylphenols in lobster hemolymph and hepatopancreas

The analysis of the hexane extracts of 14 samples of lobster hemolymph by GC/MS indicated the presence of four alkylphenols: 2-*t*-butyl-4-(dimethylbenzyl)phenol (molecular weight 268); 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)-phenol (molecular weight 324, CAS no. 34624-81-2), also named 2,6-*di-tert*-butyl-4-cumylphenol; 2,4-bis-(dimethylbenzyl)phenol (molecular weight 330, CAS no. 2772-45-4), also named 2,4-dicumylphenol; and 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol (molecular weight 386). The mass spectra for these chemicals matched those of the published library (Wiley) mass spectral database with a quality fit of more than 90%. Further confirming the identity of these compounds, the retention times of purchased or chemically synthesized standards also gave the same retention times and mass spectra as those of the compounds identified in the hemolymph (Figs. 1, 2, 3).

The levels of these four alkylphenols varied between lobsters. The compound 2-*t*-butyl-4-(dimethylbenzyl)phenol was found in 13 of the 14 lobsters analyzed, at concentrations ranging from 0.02 to 1.15 μg/ml of hemolymph, giving an average of 0.46 $\pm$ 0.11 μg/ml (mean $\pm$ standard error of the mean). The compound 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol was present in the hemolymph of 11 of the 14 lobsters, at concentrations ranging from 0.01 to 13.00 μg/ml of hemolymph, with an average of 1.89 $\pm$ 1.14 μg/ml. The compound 2,4-bis-(dimethylbenzyl)phenol was detected in the hemolymph of all 14 lobsters analyzed, at concentrations ranging from 0.07 to 19.78 μg/ml of hemolymph, giving an average of 4.03 $\pm$ 1.52 μg/ml. The compound 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol was found in the hemolymph of 11 of the 14 lobsters, at concentrations ranging from 0.20 to 70.71 μg/ml of hemolymph, giving an average of 10.98 $\pm$ 6.414 μg/ml.

The relative amounts of these four phenols varied greatly between the two localities from which the lobsters were taken (Table 1). Lobsters from Long Island Sound showed much higher average concentrations of 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, 2,4-bis-(dimethylbenzyl)phenol, and 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol than those from Vineyard Sound, whereas lobsters from Vineyard Sound had much higher average concentrations of 2-*t*-butyl-4-(dimethylbenzyl)phenol. Interestingly, the phthalate ester diisooctylphthalate was also identified in lobster hemolymph, but only in the seven lobsters analyzed from Vineyard Sound, with concentrations ranging from 0.04 to 0.39 μg/ml of hemolymph.

Two of the phenols were also found in extracts made from the hepatopancreas of two additional lobsters. The concentrations of 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol in the two hepatopancreas samples were found to be 2.55 and 6.11 μg/g of tissue, and concentrations of 2,4-bis-

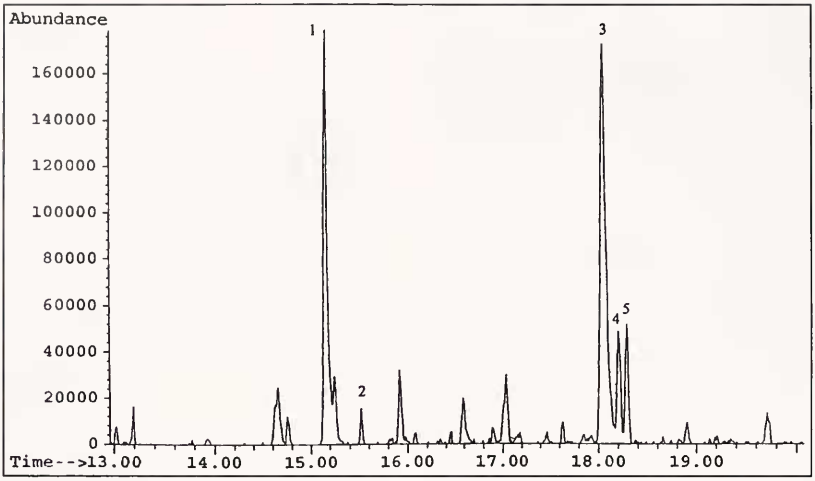

Figure 1. Gas chromatogram of a lobster hemolymph sample from Martha's Vineyard showing relative retention times of 2-*t*-butyl-4-(dimethylbenzyl)phenol, MW 268 (peak 1); 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)-phenol, MW 324 (peak 2); 2,4-bis-(dimethylbenzyl)phenol, MW 330 (peak 3); 2,4-bis(dimethylbenzyl)-6-*t*-butylphenol, MW 386 (peak 4); and diisooctylphthalate, MW 390 (peak 5).

(dimethylbenzyl)-6-*t*-butylphenol were 23.56 and 26.89 μg/g.

Alkylphenols in marine sediments

Analysis by GC/MS of ethyl ether fractions from silica columns, derived from a Vineyard Sound sediment sample and a Great Bay sediment sample, both of which showed high JH activity in the *Capitella* bioassays (data not shown), indicated the presence of the same four alkylphenols found in the lobster: 2-*t*-butyl-4-(dimethylbenzyl)phenol; 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, 2,4-bis-(dimethylbenzyl)phenol, and 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol (molecular weight 386).

The concentrations determined for these alkylphenols differed between the two sediment samples analyzed (Table 2). Concentrations of all four phenols were higher in the sediment sample from Great Bay, New Jersey (range from 21.58 to 251.01 μg/g of sediment) than in the sediment sample from Vineyard Sound, Massachusetts (range between 8.95 and 181.20 μg/g of sediment). Of considerable interest is the finding that both samples showed the same relative profiles, with 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol being found in the highest concentration of the four phenols, at 181.20 to 251.01 μg/g of sediment; followed by 2,4-bis-(dimethylbenzyl)phenol at concentrations ranging from 138.94 to 224.89 μg/g of sediment; followed by 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol ranging in concentration from 49.47 to 77.89 μg/g; and with 2-*t*-butyl-4-(dimethylbenzyl)phenol being the lowest at 8.95 to 21.58 μg/g.

JH activity of alkylphenols

One of the phenols identified, 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, was previously developed as a juvenile-hormone mimic and mosquito insecticide (MON-0585) by the Monsanto Chemical Corporation (Sacher, 1971; Jakob and Schoof, 1972), so it was of great interest to examine these phenols for juvenile-hormone activity. Results of the *Capitella* bioassay showed that the MON-0585 compound had very high JH activity (EC_{50} at 0.5 μM) compared with MF, JH I, JH III, and the JH analog pyriproxyfen, which was also developed as an insecticide (Table 3). The other three alkylphenols identified also exhibited very high JH activity. Since these compounds share a high structural homology with the known xenoestrogens bisphenol A and 4-cumylphenol (Fig. 4), these latter chemicals were also tested for JH activity. Bisphenol A showed very high JH activity (EC_{50} of 0.05 μM), whereas 4-cumylphenol showed high activity (EC_{50} of 3 μM). Mixed isomers of nonylphenol, a well-known alkylphenol, also showed high JH activity (EC_{50} of 1 μM) (Table 3).

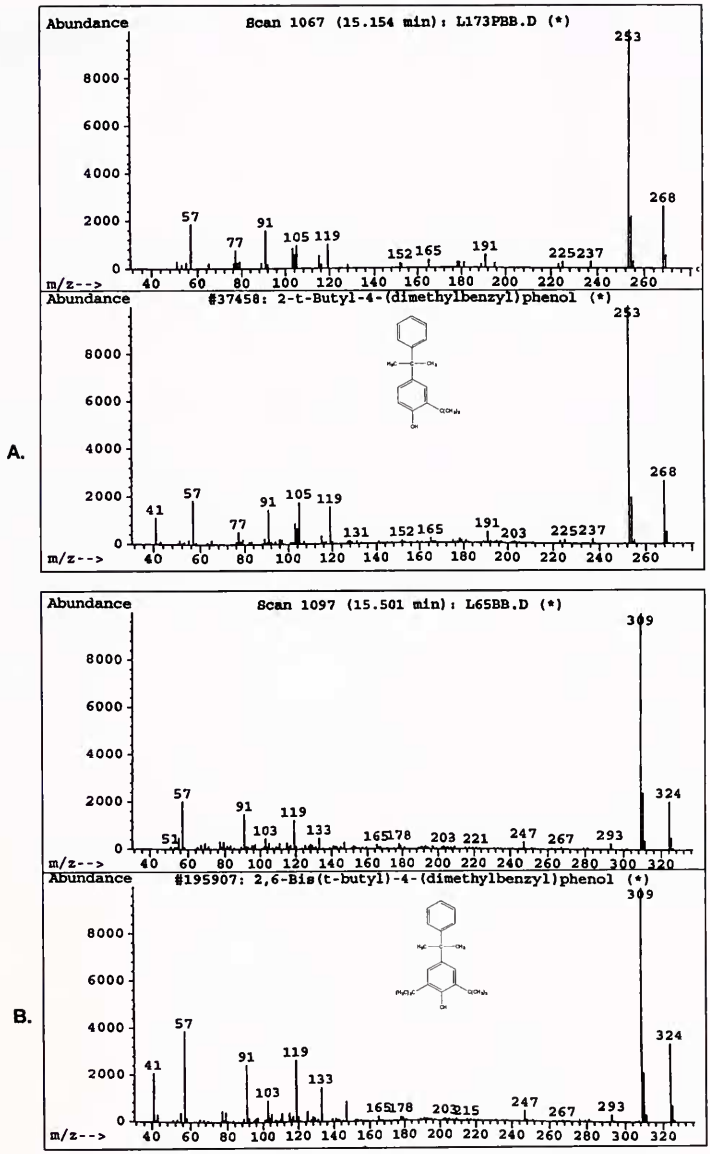

Figure 2. (A) 2-*t*-Butyl-4-(dimethylbenzyl)phenol. Mass spectra from lobster hemolymph (upper) and from reference library database, along with structure (lower) of 2-*t*-butyl-4-(dimethylbenzyl)phenol (molecular weight 286). (B) 2,6-Bis-(*t*-butyl)-4-(dimethylbenzyl)phenol. Mass spectra from lobster hemolymph (upper) and from reference library database, along with structure (lower) of 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol (molecular weight 324).

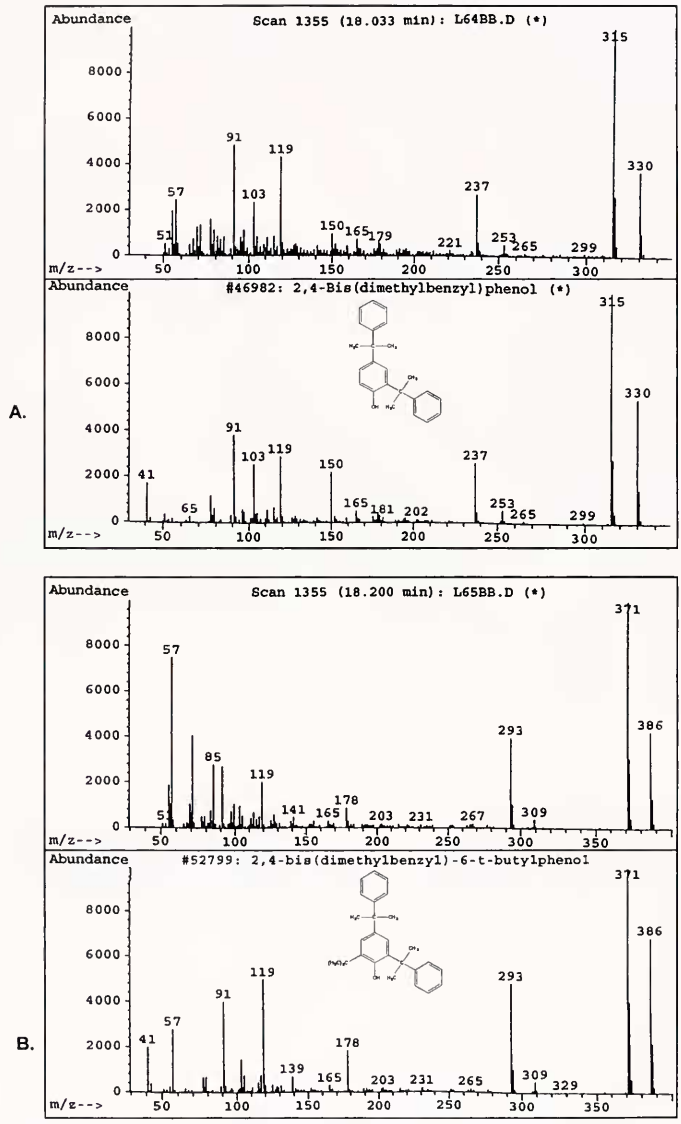

Figure 3. (A) 2,4-Bis-(dimethylbenzyl)phenol. Mass spectra from lobster hemolymph (upper) and from reference library database, along with structure (lower) of 2,4-bis-(dimethylbenzyl)phenol (molecular weight 330). (B) 2,4-Bis-(dimethylbenzyl)-6-*t*-butylphenol. Mass spectra from lobster hemolymph (upper) and from reference library database, along with structure (lower) of 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol (molecular weight 386).

Table 1

Relative concentrations of alkylphenols in hemolymph from lobsters collected from Long Island Sound (LIS) and Vineyard Sound (VS)

Alkylphenol	Average hemolymph concentration (μg/ml) ± SEM		
	LIS	VS	Combined (LIS + VS)
2-*t*-butyl-4-(dimethylbenzyl)phenol	0.10 ± 0.06	0.83 ± 0.07	0.46 ± 0.11
2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol	3.76 ± 2.11	0.03 ± 0.01	1.89 ± 1.14
2,4-bis-(dimethylbenzyl)phenol	5.17 ± 3.05	2.90 ± 0.55	4.03 ± 1.52
2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol	21.50 ± 11.89	0.45 ± 0.09	10.98 ± 6.41

SEM: standard error of the mean.
$n = 7$ for each mean for LIS and VS; $n = 14$ for combined animals.

Discussion

Our analyses by GC/MS indicate the presence of alkylphenols in 14 samples of lobster hemolymph, 2 samples of hepatopancreas tissue, and two samples of marine sediment. Alkylphenols are used primarily in the production of alkylphenol ethoxylates (APEs), which are found in industrial and household detergents, surfactants, paints, and wetting agents, and which have applications in wood pulping, textile manufacture, plastics manufacture, and petroleum recovery, among other uses (Naylor *et al.*, 1992). Besides their use to produce APEs, alkylphenols are also used in the production of phenolic resins, as antioxidant stabilizers for plastics and polymers, and as curing agents (Ying *et al.*, 2002).

An estimated 500 million pounds of alkylphenols are used annually (Zintek *et al.*, 2003), and an estimated 500,000 tons of APEs are produced annually (Naylor *et al.*, 1992; Ying *et al.*, 2002). Environmental contamination by these chemicals and their breakdown products in rivers, oceans, and sediments is well known and widespread (Hale *et al.*, 2000). Of the 500,000 tons of APEs produced, about 60 percent are estimated to end up in the aquatic environment, as these chemicals and their breakdown products (which are alkylphenols) are released from wastewater outfalls or directly into the environment (Renner, 1997; Ying *et al.*, 2002). Alkylphenols have been detected in the water, in sediments, and in fish tissues (Lye *et al.*, 1999; van Heemst *et al.*, 1999); in sediments, levels have been reported to be as high as 70 μg/g in the United States (Ying *et al.*, 2002).

The alkylphenols identified in this report are similar in structure to bisphenol A (BPA), a well-known endocrine disruptor (Fig. 4). BPA is utilized primarily in the production of polycarbonate plastics. It is also a major antioxidant component of the epoxy resins used to line food cans and pipes, and is used in dental sealants (Staples *et al.*, 1998). Over 200,000 tons of BPA are produced annually by Japan

Table 2

Relative concentrations of alkylphenols in marine sediments from Vineyard Sound, Massachusetts (VS) and Great Bay, New Jersey (GB)

Alkylphenol	Concentration (μg/gm sediment)	
	VS	GB
2-*t*-butyl-4-(dimethylbenzyl)phenol	8.95	21.58
2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol	49.47	77.89
2,4-bis-(dimethylbenzyl)phenol	138.94	224.89
2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol	181.20	251.05

Data shown is for one sediment sample from VS and one from GB.

Table 3

Juvenile hormone activity of alkylphenols compared with activity of known juvenile hormones, using a Capitella *settlement and metamorphosis bioassay*

Chemical tested	EC_{50} (μM)
Juvenile hormones	
JH I	25
JH III	3
trans, trans-methyl farnesoate (crustacean juvenile hormone)	1
pyriproxyfen (JH-mimic)	8
Alkylphenols	
2-*t*-butyl-4-(dimethylbenzyl)phenol	1
2,4-bis-(dimethylbenzyl)phenol	2
2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol (JH-mimic MON-0585)	0.5
2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol	1
4-cumylphenol	3
bisphenol A	0.05
nonylphenol (mixed isomers)	1
Other chemicals tested	
farnesol	40
arachidonic acid	70
stearic acid	410
cholesterol	NA
lutein	NA

NA: not active at highest concentration tested (1000 μM).

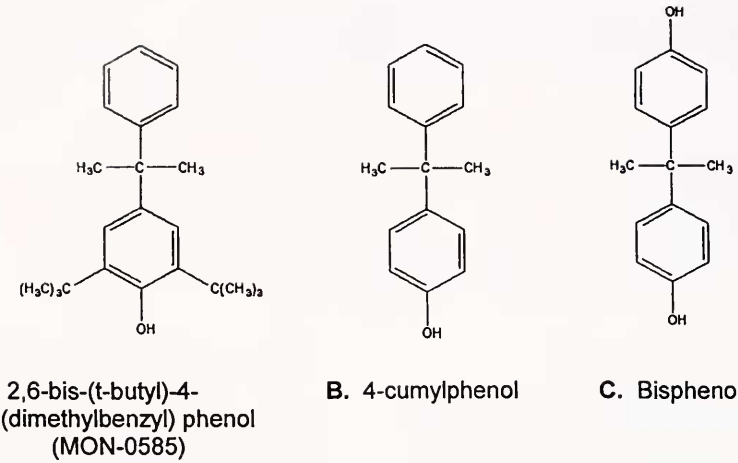

Figure 4. Comparison of chemical structures of 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol, 4-dimethylbenzylphenol, and bisphenol A. (A) Chemical structure of the juvenile hormone mimic and mosquito insecticide MON-0585 (same as 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol). (B) Chemical structure of 4-cumylphenol. (C) Chemical structure of the known endocrine disruptor bisphenol A.

alone (Kamiura *et al.*, 1997), and environmental contamination by this chemical has in recent years been of major public concern. The endocrine-disrupting effects of BPA have been demonstrated to alter the reproductive physiology and development of mammals (Stoker *et al.*, 1999; Takao *et al.*, 1999), fish, and invertebrates—including molting of the insect *Chironomus riparius* (Watts *et al.*, 2001; Segner *et al.*, 2003). BPA can leach from food cans and plastic bottles into foods and beverages and from there into the human digestive system; it subsequently travels through sewage treatment plants and eventually into river systems and oceans (Staples *et al.*, 1998; Fromme *et al.*, 2002). Furthermore, the plastic-particle waste that is prevalent in the oceans can also directly leach BPA into the environment (Sajiki and Yonekubo, 2003). BPA contamination in sediments is widespread; for example, levels have been reported as 0.05 μg/g dry weight in Ulsan Bay, Korea (Khim *et al.*, 2001), in Onsan Bay, Korea; as 0.20 μg/g dry weight (Koh *et al.*, 2002); and as 0.19 μg/g in Germany (Fromme *et al.*, 2002).

The four alkylphenols we have identified in lobsters and marine sediments are used together in antioxidant blend formulations for manufacturing rubber and plastic polymers. A patent by Russell *et al.* (2002) states that these phenols are found in the Wingstay C and Polystay C antioxidant formulations used in tire manufacturing by the Goodyear Tire and Rubber Co. Similarly, a patent by Messina *et al.* (1982) states that these phenols are added as stabilizers for organic polymers including rubbers and plastics. Other antioxidant applications include their use in pesticide formulations and in therapeutics (Smith, 2002). These phenolic antioxidants therefore appear to be widely employed in a fashion similar to BPA.

2,4-bis-(dimethylbenzyl)phenol

One alkylphenol we found in lobster hemolymph and marine sediments, 2,4-bis-(dimethylbenzyl)phenol, is sold under the tradename 2,4-dicumylphenol, or 2,4-DCP. This reagent is used in antioxidant mixtures (Messina *et al.*, 1982; Russell *et al.*, 2002) as previously mentioned, and appears to also have a use in surfactant formulations. This chemical is also released into the environment upon hydrolysis of the antioxidant plasticizer bis-(2,4-dicumylphenyl)pentaerythritol diphosphite. The use of this plasticizer in food containers is regulated by the food industry (Scientific Committee on Food, 2001). The 2,4-DCP compound is close in structure to that of 4-cumylphenol, another industrial alkylphenol (Fig. 4). Hale *et al.* (2000) reported levels of 4-cumylphenol as high as 70 μg/g of sediment in sediments near wastewater outfalls in the United States. To our knowledge, pollution by 2,4-bis-(dimethylbenzyl)phenol in sediments or aquatic life has not been reported. However, we found this phenol in sediments from Vineyard Sound at a concentration of 138.94 μg/g (wet weight) and at higher concentrations in Great Bay, New Jersey, at 224.89 μg/g, which is close to the concentration reported for 4-cumylphenol by Hale *et al.* (2000).

2,6-bis-(t-butyl)-4-(dimethylbenzyl)phenol

Concentrations of 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)-phenol were higher in lobsters taken from Long Island Sound than in those from Vineyard Sound, and this phenol was also found in sediment samples from the two locations. As previously mentioned, Monsanto developed this compound as a juvenile hormone mimic, named MON-0585, for application as a mosquito insecticide (Sacher, 1971; Jakob and Schoof, 1972); however, it was supposedly never brought to commercial use (Schaefer *et al.,* 1974). Like other alkylphenols and BPA, this one has also gained industrial use as an antioxidant in polymer manufacture (Hanauye *et al.,* 1976; Messina *et al.,* 1982; Russell *et al.,* 2002). Environmental contamination by this chemical has presumably not been documented before; however, it has been found in propolis, which is produced by bees and derived from the resins of tree bark and leaves (Hegazi and El Hady, 2002).

2-t-butyl-4-(dimethylbenzyl)phenol and 2,4-bis-(dimethylbenzyl)-6-t-butylphenol

The compounds 2-*t*-butyl-4-(dimethylbenzyl)phenol and 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol were both found in lobster hemolymph and marine sediments, and are used industrially in antioxidant blends for the manufacturing of rubber and other polymers (Messina *et al.,* 1982; Russell *et al.,* 2002). Interestingly, these phenols, along with 2,4-bis-(dimethylbenzyl)phenol, were found to be cyclooxgenase inhibitors that occur naturally in peat (Russell *et al.,* 2002). Under the tradename isobutylenated methylstyrenated phenol, the 2,4-bis-(dimethylbenzyl)-6-*t*-butylphenol compound is listed as having a high production volume (more than a million pounds produced per year) by the U.S. Environmental Protection Agency in its High Production Volume Challenge Program, which encourages manufacturers to investigate the toxicity of these chemicals (U.S. EPA, 2002). This compound has also been reported in sediments contaminated with coal tar sediments (Zeng and Hong, 2002). The 2-*t*-butyl-4-(dimethylbenzyl)phenol compound, however, has not been reported as a contaminant until now.

Probable sources of the alklphenols and likelihood of the presence of other phenols

Given that these identified alkylphenols are used in industrial antioxidant formulations similar to those of BPA, and that alkylphenol and BPA contamination is well known and widespread, it is likely that the source of these identified phenols is alkylphenol contamination originating from wastewater outfalls or released directly into the environment; these are the sources that have been identified for other alkylphenols and BPA (Ying *et al.,* 2002). Surface runoff from heavily traveled roadways containing tire residue may be a contributing source of these chemicals. It is to be noted, however, that three of these compounds were also found naturally in peat bog material (Russell *et al.,* 2002), indicating that they may be residues derived from breakdown of plant material. More research is needed to determine the sources of these chemicals. It should also be emphasized that the nonpolar extraction method employed, using hexane, may not be suitable for the extraction of some of the more polar phenols, such as BPA and 4-dimethylbenzylphenol, which may therefore also be present.

In support of the view that environmental contamination is a source for alkylphenols, the plasticizer diisooctylphthalate was also found in fairly high concentrations in the hemolymph of 7 of the 14 lobsters examined, indicating these lobsters indeed had exposure to plasticizers. Control experiments done in our laboratory showed that the phenols and diisooctylphthalate found were not derived from laboratory contamination by soap, glassware, pipettes, or syringes, and were not from the GC columns since control extractions did not produce these chemicals. It therefore appears that these alkylphenols, like other alkylphenols and BPA, result from environmental contamination. The relative levels of these phenols in the lobsters and sediments differed at different locations, and this presumably reflects different amounts and different formulations of alkylphenols used in different geographic areas. Alkylphenols may also have originated from nonlocal sources and been carried by currents.

Effects of alkylphenols on crustaceans

What effect these compounds have on lobsters is currently being investigated in our laboratory. Given that these phenols are similar in structure to BPA and show high JH activity in bioassays, it is likely that they have serious toxic and endocrine-disrupting effects. The high toxicity of alkyphenols to aquatic life has recently been documented by the U.S. Environmental Protection Agency, as has the fact that these chemicals persist in the environment, including sediments (U.S. EPA, 2003). Indeed, toxicity studies with 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol (MON-0585) have shown that this compound does affect nontarget crustaceans (reviewed by Williams and Duke, 1979). In experiments by Costlow (1977), megalopa larvae of the blue crab *Callinectes sapidus* were all killed in water containing 10 ppm MON-0585, and 60% were killed in 1 ppm MON-0585. Sublethal behavioral effects of MON-0585 on the swimming speed and phototaxis of larvae of the crab *Rhithropanopeus harrisii* have also been reported (Forward and Costlow, 1976) and attributed to the effects of JH mimics on increased respiration (Slama and Kryspin, 1979). Thus lobster larval development and metamorphosis are likely to be affected by this compound at critical concentrations. The other three phenols found probably also have effects on

lobster, since they also exhibit high JH activity and are structurally related to MON-0585 and BPA. For example, 4-nonylphenol is acutely toxic to lobsters, with an LC_{50} in seawater reported as 0.2 ppm (0.2 μg/ml) (Cox, 1996), and has been found to affect the development of other crustaceans, including barnacles, in which it inhibits settlement and induces synthesis of vitellin-like proteins (Billinghurst *et al.,* 1998, 2000). In our quantitative determinations, we found levels of 2,6-bis-(*t*-butyl)-4-(dimethylbenzyl)phenol (MON-0585) in the hemolymph of lobsters as high as 13 ppm, which Costlow (1977) found to be a lethal external concentration for crab larvae. The evidence thus indicates that these phenols may be contributing significantly to the lobster deaths seen in Long Island Sound, particularly under stressful environmental conditions such as high temperatures and hypoxia. Since JH mimics such as MON-0585 can increase respiration in insects (Slama and Kryspin, 1979) and possibly crustaceans (Forward and Costlow, 1976), these phenols may make the lobsters more susceptible to stress at low levels of oxygen. Furthermore, MON-0585 has been found to inhibit cuticle arylation and hardening in mosquitoes (Zomer and Lipke, 1981; Semensi and Sugumaran, 1986). We speculate that this compound may also interfere with cuticle formation and hardening in lobsters, making them more susceptible to chitinolytic microorganisms and shell disease. This disease has become increasingly prevalent in lobsters in recent years (Castro and Angell, 2000).

Because MON-0585 was developed as a juvenile hormone mimic, it is not surprising that other structurally related alkylphenols also possess JH activity: all four alkylphenols exhibited such activity in the *Capitella* bioassay when tested at concentrations found in the hemolymph (Tables 2, 3), raising the possibility that these alkylphenols may have JH-like effects on the lobster. Because reproduction, development, and metamorphosis in crustaceans are partly regulated by methyl farnesoate, a compound with juvenile hormone activity, the alkylphenols we investigated may function as endocrine disruptors in the lobster at low concentrations. Exogenous application of JH analogs can perturb normal metamorphosis and molting (reviewed by Laufer and Biggers, 2001). Like JH and its analogs, the JH-active alkylphenols may act through membrane-bound, intracellular, and nuclear receptors to bring about changes in morphogenesis and stimulation of vitellogenesis through vitellogenin gene induction and increased vitellogenin uptake (Engelmann, 1983; Sehnal, 1983; Wyatt, 1991; Davey and Gordon, 1996; Jones and Sharp, 1997). As evidence for this mechanism of action, both 4-nonylphenol and BPA have been found to induce vitellogenesis in vertebrates (Jones *et al.,* 2000).

To our knowledge, our results are the first to demonstrate that xenoestrogens such as BPA and nonylphenol have JH activity, indicating a possible relationship between estrogenicity and juvenile hormone activity, and the further possibility that estrogens and juvenile hormones share similar mechanisms of action.

Possible bioaccumulation and health effects of the identified alkylphenols

The presence of phenolic compounds in marine sediments suggests that lobsters may acquire them through the food chain as found for other polyaromatic hydrocarbons (Pruell *et al.,* 2000). Interestingly, in the report by Hale *et al.* (2000), levels of 4-cumylphenol in sediments was found to be much higher than those of 4-nonylphenol (70,000 μg/kg compared with 11,000 μg/kg), even though 80% of the alkylphenols used in formulations are nonylphenol ethoxylates (Ying *et al.,* 2002). This suggests that polyaromatic alkylphenols are more recalcitrant to biodegradation, as would be expected. It is therefore likely that, due to their polyaromatic structure, the alkylphenols we identified in the sediments are also more resistant to degradation and may accumulate in sediments and benthic invertebrates. These alkylphenols were also found in lobster hepatopancreas, and may be bioaccumulated there, as other polyaromatic hydrocarbons are known to be (McLeese and Metcalfe, 1979; James *et al.,* 1995). Bioaccumulation in seafoods has been documented for 4-nonylphenol (Ekelund *et al.,* 1990; Ferrara *et al.,* 2001). This raises concern for human health. Since 1995, the European community has placed a voluntary ban on the use of alkylphenol ethoxylates (APEs) due to the toxicity and endocrine-disruptor activity of the breakdown products (including 4-nonylphenol and 4-cumylphenol); use of these products has not been banned in the United States (Renner, 1997). This has sparked considerable debate among researchers and regulators. The potentially endocrine-disrupting effects of BPA have raised particular health concerns, especially since these are viewed as being potentially carcinogenic to humans (reviewed by Cox, 1996, and Lathers, 2002). Since the compounds identified are similar in structure to BPA, the presence of these compounds in lobsters may also warrant health concerns.

Acknowledgments

We acknowledge the help of Dr. Mike Syslo of the Massachusetts Division of Marine Fisheries lobster hatchery on Martha's Vineyard and Mrs. Penny Howell of the Connecticut Department of Environmental Protection for kindly supplying lobsters for this study. We also acknowledge Dr. Judith Grassle, Rutgers University, for kindly supplying marine sediment samples and *Capitella* stocks, and Dr. William Bowers, University of Arizona, for kindly supplying pyriproxyfen. In addition, we are grateful to Professor James M. Bobbitt, Professor James D. Stuart, and Mr. Christopher P. Capulong, of the Department of Chemistry, University of Connecticut, for helpful discussions on

chemical synthesis and analysis. We also acknowledge the help and expertise of Mr. Marvin Thompson, manager of the mass spectrometry laboratory and the Department of Chemistry, University of Connecticut, for use of the GC/MS facilities. We gratefully acknowledge the Sea Grant College Program, NOAA, and the Connecticut Department of Environmental Protection for providing financial support for this research.

Literature Cited

Biggers, W. J. 1994. Effects of juvenile hormone-active compounds on larval settlement and metamorphosis of the polychaete annelid *Capitella* Sp. 1. Ph.D. dissertation, University of Connecticut.

Biggers, W. J., and H. Laufer. 1992. Chemical induction of settlement and metamorphosis of *Capitella capitata* sp. 1. (Polychaetea) larvae by juvenile hormone-active compounds. *Invertebr. Reprod. Dev.* **22:** 39–46.

Biggers, W. J., and H. Laufer. 1996. Detection of juvenile hormone-active compounds by larvae of the marine annelid *Capitella* sp. 1. *Arch. Insect Biochem. Physiol.* **32:** 475–484.

Billinghurst, Z., A. S. Clare, T. Fileman, J. McEvoy, J. Readman, and M. H. Depledge. 1998. Inhibition of barnacle settlement by the environmental oestrogen 4-nonylphenol and the natural oestrogen 17β-oestradiol. *Mar. Pollut. Bull.* **36:** 833–839.

Billinghurst, Z., A. S. Clare, K. Matsumura, and M. H. Depledge. 2000. Induction of cypris major protein in barnacle larvae by exposure to 4-n-nonylphenol and 17β-oestradiol. *Aquat. Toxicol.* **47:** 203–212.

Castro, K. M., and T. E. Angell. 2000. Prevalence and progression of shell disease in American lobster, *Homarus americanus*, from Rhode Island waters and the offshore canyons. *J. Shellfish Res.* **19:** 691–700.

Costlow, J. D. 1977. The effect of juvenile hormone mimics on development of the mud crab *Rhithropanopeus harrisii* (Gould). Pp. 439–457 in *Physiological Responses of Marine Biota to Pollutants*, F. J. Vernberg, A. Calabrese, F. P. Thurberg, and W. B. Vernberg, eds. Academic Press, New York.

Cox, C. 1996. Nonylphenol and related chemicals. *J. Pest. Reform* [Online] **16:** 15–20. Available: http://www.pesticide.org/nonyl.pdf [2003, 29 September].

Davey, K. G., and D. R. B. Gordon. 1996. Fenoxycarb and thyroid hormones have JH-like effects on follicle cells of *Locusta migratoria* in vitro. *Arch. Insect Biochem. Physiol.* 613–622.

Ekelund, R., A. Bergman, A. Granmo, and M. Berggren. 1990. Bioaccumulation of 4-nonylphenol in marine animals: a re-evaluation. *Environ. Pollut.* **64:** 107–120.

Engelmann, F. 1983. Vitellogenesis controlled by juvenile hormone. Pp. 259–270 in *Invertebrate Endocrinology, Vol. 1, Endocrinology of Insects*, R. G. H. Downer and H. Laufer, eds. A. R. Liss, New York.

Ferrara, F., F. Fabietti, M. Delise, A. P. Bocca, and E. Funari. 2001. Alkylphenolic compounds in edible molluscs of the Adriatic Sea (Italy). *Environ. Sci. Technol.* **35:** 3109–3112.

Forward, R. B., Jr., and J. D. Costlow, Jr. 1976. Crustacean larval behavior as an indicator of sublethal effects of an insect juvenile hormone mimic. Pp. 279–289 in *Estuarine Processes, Vol. 1, Uses, Stresses and Adaptation to the Estuary*, M. Wiley, ed. Academic Press, New York.

Frommie, H., T. Kuechler, T. Otto, K. Pilz, J. Mueller, and A. Wenzel. 2002. Occurrence of phthalates and bisphenol A and F in the environment. *Water Res.* **36:** 1429–1438.

Hale, R. C., C. L. Smith, P. DeFur, E. Harvey, E. Bush, M. J. LaGuardia, and G. G. Vadas. 2000. Nonylphenols in sediments and effluents associated with diverse wastewater outfalls. *Environ. Toxicol. Chem.* **19:** 946–952.

Hanauye, K., T. Takase, M. Iwasa, and M. Ito. 1976. Method for producing 2,6-di-tert.-butyl-4-cumyl phenol. *United States Patent* No. 3,933,928. [Online] Available: www.uspto.gov [2003, September 29].

Hegazi, A. G., and F. K. A. El Hady. 2002. Egyptian propolis: 3. Antioxidant, antimicrobial activities and chemical composition of propolis from reclaimed lands. *Z. Naturforsch. Sect. C* **57:** 395–402.

Jakub, W. L., and H. F. Schoof. 1972. Mosquito larvicide studies with MON 585, a juvenile hormone mimic. *Mosq. News* **32:** 6–10.

James, M. O., A. H. Altman, C. L. J. Li, and J. D. Schell. 1995. Biotransformation, hepatopancreas DNA binding and pharmacokinetics of benzo(a)pyrene after oral and parenteral administration to the American lobster, *Homarus americanus*. *Chem-Biol. Interact.* **95:** 141–160.

Jones, G., and P. A. Sharp. 1997. Ultraspiracle: an invertebrate nuclear receptor for juvenile hormones. *Proc. Natl. Acad. Sci. USA* **94:** 13499–13503.

Jones, P. D., W. M. DeCoen, L. Tremblay, and J. P. Giesy. 2000. Vitellogenin as a biomarker for environmental estrogens. *Water Sci. Technol.* **42:** 1–14.

Kamiura, T., Y. Tajima, and T. Nakahara. 1997. Determination of bisphenol A in air. *J. Environ. Chem.* **7:** 275–279.

Khim, J. S., K. T. Lee, K. Kannan, D. L. Villeneuve, J. P. Giesy, and C. H. Koh. 2001. Trace organic contaminants in sediment and water from Ulsan Bay and its vicinity, Korea. *Arch. Environ. Contam. Toxicol.* **40:** 141–150.

Koh, C. H., J. S. Khim, D. L. Villeneuve, K. Kannan, and J. P. Giesy. 2002. Analysis of trace organic contaminants in sediment, pore water, and water samples from Onsan Bay, Korea: instrumental analysis and *in vitro* gene expression assay. *Environ. Toxicol. Chem.* **21:** 1796–1803.

Lathers, C. M. 2002. Endocrine disruptors: a new scientific role for clinical pharmacologists? Impact on human health, wildlife, and the environment. *J. Clin. Pharmacol.* **42:** 7–23.

Laufer, H., and W. J. Biggers. 2001. Unifying concepts learned from methyl farnesoate for invertebrate reproduction and post-embryonic development. *Am. Zool.* **41:** 442–457.

Long Island Sound Lobster Health Symposium. 2003. *Symposium of the Long Island Sound Lobster Research Initiative and Connecticut Department of Environmental Protection*, CT-SG-03-03, N. C. Balcom, ed. Connecticut Sea Grant College Program, NOAA.

Lye, C. M., C. L. J. Frid, M. E. Gill, D. W. Cooper, and D. M. Jones. 1999. Estrogenic alkylphenols in fish tissues, sediments, and waters from the U.K. Tyne and Tees estuaries. *Environ. Sci. Technol.* **33:** 1009–1014.

McLeese, D. W., and C. D. Metcalfe. 1979. Toxicity of creosote to larval and adult lobsters *Homarus americanus* and *Crangon septemspinosa* and its accumulation in lobster hepatopancreas. *Bull. Environ. Contam. Toxicol.* **22:** 796–799.

Messina, G., L. Lorenzoni, and A. Fadda. 1982. Phenolic antioxidant compositions for organic polymers. *United States Patent* No. 4,439,570. [Online] Available: www.uspto.gov [2003, September 29].

Naylor, C. G., J. P. Mieure, W. J. Adams, J. A. Weeks, F. J. Castaldi, L. D. Ogle, and R. R. Romano. 1992. Alkylphenol ethoxylates in the environment. *J. Am. Oil Chem. Soc.* **69:** 695–703.

Pruell, R. J., B. K. Taplin, D. G. McGovern, R. McKinney, and S. B. Norton. 2000. Organic contaminant distributions in sediments, polychaetes (*Nereis virens*) and American lobster (*Homarus americanus*) from a laboratory food chain experiment. *Mar. Environ. Res.* **49:** 19–36.

Renner, R. 1997. European bans on surfactant trigger transatlantic debate. *Environ. Sci. Technol.* **31:** 316A–320A. [Online] July 1997. Available: http://pubs.acs.org/hotartcl/est/97/jul/euro.html [2003, September 29].

Russell, B. A., J. D. Miller, J. R. Cashman, and S. A. Weerawarna.

2002. Naturally occurring compounds and their derivatives as cyclooxygenase 2 and/or 5-lipoxygenase inhibitors. *United States Patent* No. 6,365,634. [Online] Available: www.uspto.gov [2003, September 29].

Sacher, R. 1971. A mosquito larvicide with favorable environmental properties. *Mosq. News* **31:** 513–516.

Sajiki, J., and J. Yonekubo. 2003. Leaching of bisphenol A (BPA) to seawater from polycarbonate plastic and its degradation by reactive oxygen species. *Chemosphere* **51:** 55–62.

Schaefer, C. H., T. Miura, F. S. Mulligan, III, and R. M. Takahashi. 1974. Insect development inhibitors: biological activity of RE17656, RE17937, and RE18286 against mosquitoes (Diptera:Culicidae). *Proc. Pap. Ann. Conf. Calif. Mosq. Control Assoc.* **42:** 147–151.

Scientific Committee on Food. 2001. Opinion of the Scientific Committee on Food on the 12th additional list of monomers and additives for food contact materials. Report CS/PM/GEN/M84. European Commission, Health and Consumer Protection Directorate-General, Brussels. [Online] Available: http://europa.eu.int/comm/food/fs/sc/scf/out84_en.pdf [2003, October 20].

Segner, H., K. Caroll, M. Fenske, C. R. Janssen, G. Maack, D. Pascoe, C. Schafers, G. F. Vandenbergh, M. Watts, and A. Wenzel. 2003. Identification of endocrine disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. *Ecotoxicol. Environ. Saf.* **54:** 302–314.

Sehnal, F. 1983. Juvenile hormone analogues. Pp. 657–672 in *Invertebrate Endocrinology, Vol. 1, Endocrinology of Insects,* R. G. H. Downer and H. Laufer, eds. A. R. Liss, New York.

Semensi, V., and M. Sugumaran. 1986. Effect of MON-0585 on sclerotization of *Aedes aegypti* cuticle. *Pestic. Biochem. Physiol.* **26:** 220–230.

Slama, K., and S. I. Kryspin. 1979. Hypermetabolic response induced by juvenile hormone analogs in an insect. *Z. Naturforsch. Biosci.* **34:** 599–607.

Smith, J. B. 2002. Zinc containing compositions for anti-viral use. *United States Patent* No. 6,475,526. [Online] Available: www.uspto.gov [2003, September 29].

Staples, C. A., P. B. Dom, G. M. Klecka, S. T. O'Block, and L. R. Harris. 1998. A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere* **36:** 2149–2173.

Stoker, T. E., C. L. Robinette, B. H. Britt, S. C. Laws, and R. L. Cooper. 1999. Prepubertal exposure to compounds that increase prolactin secretion in the male rat: effects on the adult prostate. *Biol. Reprod.* **61:** 1636–1643.

Takan, T., W. Nanamiya, I. Nagano, K. Asaba, K. Kawabata, and K. Hashimoto. 1999. Exposure with the environmental estrogen bisphenol A disrupts the male reproductive tract in young mice. *Life Sciences* **65:** 2351–2357.

U.S. EPA (U.S. Environmental Protection Agency). 2002. High Production Volume (HPV) Challenge Program. Robust summaries and test plans: hindered phenols. EPA comments, American Chemistry Council. [Online] Available: http://www.epa.gov/chemrtk/hndrdphn/c13382tc.htm [2003, September 29].

U.S. EPA (U.S. Environmental Protection Agency). 2003. Design for the Environment. Key characteristics of laundry detergent ingredients. [Online] Available: http://www.epa.gov/dfe/pubs/laundry/techfact/keychar.htm [2003, September 29].

van Heemst, J. D. H., P. F. van Bergen, B. A. Stankiewicz, and J. W. de Leeuw. 1999. Multiple sources of alkylphenols produced upon pyrolysis of DOM, POM and recent sediments. *J. Anal. Appl. Pyrolysis* **52:** 239–256.

Watts, M. W., D. Pascoe, and K. Carroll. 2001. Chronic exposure to 17-alpha-ethinylestradiol and bisphenol A-effects on development and reproduction in the freshwater invertebrate *Chironomus riparius* (Diptera: Chironomidae). *Aquat. Toxicol.* **55:** 113–124.

Williams, A. B., and T. W. Duke. 1979. Crabs (Arthropoda: Crustacea: Decapoda: Brachyura). Pp. 171–233 in *Pollution Ecology of Estuarine Invertebrates,* C. W. Hart and L. H. Samuel, eds. Academic Press, New York.

Wyatt, G. R. 1991. Gene regulation in insect reproduction. *Invertebr. Reprod. Dev.* **20:** 1–35.

Ying, G.-G., B. Williams, and R. Kookana. 2002. Environmental fate of alkylphenols and alkylphenol ethoxylates—a review. *Environ. Int.* **28:** 215–226.

Zeng, Y., and P. K. A. Hong. 2002. Slurry-phase ozonation for remediation of sediments contaminated by polycyclic aromatic hydrocarbons. *J. Air Waste Manag. Assoc.* **52:** 58–68.

Zintek, L., D. Wesolowski, B. Paruchuri, P. Howe, A. Alwan, G. Azevedo, D. Hopkins, and J. Dorkin. 2003. Methods for determination of selected alkylphenols and bisphenol A in sediments and water. EPA Conference on Analysis of Pollutants in the Environment, 26th annual conference, Chicago, IL. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division, Washington, DC.

Zomer, E., and H. Lipke. 1981. Tyrosine metabolism in *Aedes aegypti* II Arrest of sclerotization by MON 0585 and diflubenzuron. *Pestic. Biochem. Physiol.* **16:** 28–37.